Cell Meter™ Live Cell Caspase 3/7 Binding Assay Kit *Red Fluorescence*

Catalog number: 20101
Unit size: 25 Tests

Component	Storage	Amount
Component A: TF3-DEVD-FMK	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Washing Buffer	Freeze (<-15 °C), Minimize light exposure	1 bottle (100 mL)
Component C: 500X Nuclear Green™ DCS1	Freeze (<-15 °C), Minimize light exposure	1 vial (100 µL)
Component D: 500X Hoechst	Freeze (<-15 °C), Minimize light exposure	1 vial (100 µL)

OVERVIEW

Our Cell Meter™ live cell caspases activity assay kits are based on fluorescent FMK inhibitors of caspases. These inhibitors are cell permeable and non-cytotoxic. Once inside the cell, the caspase inhibitors bind covalently to the active caspases. The activation of caspase 3/7 is important for the initiation of apoptosis. It has been proven that caspase 3/7 has substrate selectivity for the peptide sequence Asp-Glu-Val-Asp (DEVD). This kit uses TF3-DEVD-FMK as a fluorescent indicator for caspase 3/7 activity. TF3-DEVD-FMK irreversibly binds to activated caspase 3/7 in apoptotic cells. Once bound to caspase 3/7, the fluorescent reagent is retained inside the cell. The binding event inhibits caspase 3/7 but will not stop apoptosis from proceeding. There are a variety of parameters that can be used for monitoring cell apoptosis. This Cell Meter™ Live Cell Caspase 3/7 Activity Assay Kit is designed to detect cell apoptosis by measuring caspase 3/7 activation in live cells. It is used for the quantification of activated caspase 3/7 activities in apoptotic cells, or for screening caspase 3/7 inhibitors. TF3-DEVD-FMK, the red label reagent, allows for direct detection of activated caspase 3/7 in apoptotic cells by fluorescence microscopy, flow cytometer, or fluorescent microplate reader. The kit provides all the essential components with an optimized assay protocol.

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds at a density of 5 × 10^5 to 2 × 10^6 cells/mL
2. Add TF3-DEVD-FMK into cell solution at 1:150 ratio
3. Incubate at room temperature for 1 hour
4. Pellet the cells, wash and resuspend the cells with buffer or growth medium
5. Monitor fluorescence intensity (bottom read mode) at Ex/Em = 550/595 nm (Cutoff = 570 nm), fluorescence microscope with TRITC filter, or flow cytometer with FL1 channel

Important Thaw all the components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microscope
Excitation:	TRITC channel
Emission:	TRITC channel
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	FITC channel for Nuclear Green™ DCS1 staining, DAPI channel for Hoechst staining

Instrument:	Flow cytometer
Excitation:	550 nm
Emission:	595 nm
Instrument specification(s):	FL1 Channel

Instrument:	Fluorescence microplate reader
Excitation:	550 nm
Emission:	595 nm
Cutoff:	570 nm
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	Bottom read mode

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. TF3-DEVD-FMK stock solution (150X):
Add 50 µL of DMSO into the vial of TF3-DEVD-FMK (Component A) to make 150X TF3-DEVD-FMK stock solution.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

1. Culture cells to a density optimal for apoptosis induction according to your specific induction protocol, but not to exceed 2 x 10^6 cells/ mL. At the same time, culture a non-induced negative control cell population at the same density as the induced population for every labeling condition. Here are a few examples for inducing apoptosis in suspension culture:

   a. Treating Jurkat cells with 2 µg/ml camptothecin for 3 hours.

   b. Treating Jurkat cells with 1 µM staurosporine for 3 hours.

   c. Treating HL-60 cells with 4 µg/ml camptothecin for 4 hours.

   d. Treating HL-60 cells with 1 µM staurosporine for 4 hours.

   Note Each cell line should be evaluated on an individual basis to determine the optimal cell density for apoptosis induction.

2. Add 150X TF3-DEVD-FMK stock solution into the cell solution at a 1:150 ratio, and incubate the cells in a 37°C, 5% CO_2 incubator for 1 hour.

   Note The cells can be concentrated up to ~ 5 X 10^6 cells/mL for TF3-DEVD-FMK labeling. For adherent cells, gently lift the cells with 0.5 mM EDTA to keep the cells intact, and wash the cells once with serum-containing media prior to incubation with TF3-DEVD-FMK. The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.

3. Spin down the cells at ~ 200g for 5 minutes, and wash cells with 1 mL Washing Buffer (Component B) twice. Resuspend the cells in desired amount of washing buffer.

   Note TF3-DEVD-FMK is fluorescent, thus it is important to wash out any unbound reagent to eliminate the background. For detached cells, the concentration of cells should be adjusted to 2 - 5 X 10^5 cells/100 µL aliquot per microtiter plate well.

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4. If desired, label the cells with a DNA stain (such as Nuclear Green™ DCS1 for dead cells, or Hoechst for whole population of the cell nucleus stain).

5. Monitor the fluorescence intensity by fluorescence microscopy, flow cytometer, or fluorescence microplate reader at Ex/Em = 550/595 nm (for Nuclear Green™ DCS1, Ex/Em = 490/525 nm, for Hoechst dyes, Ex/Em = 350/461 nm).

   For flow cytometry: Monitor the fluorescence intensity using the channel with Ex/Em = 550/595 nm (FL1 channel for Nuclear Green™ DCS1 staining). Gate on the cells of interest, excluding debris.

   For fluorescence microscope: Place 100 µL of the cell suspensions into each of wells of a 96-well black microtiter plate. Observe cells under a fluorescence microscope using TRITC channel (FITC channel for Nuclear Green™ DCS1 staining, DAPI channel for Hoechst staining).

   For fluorescence microplate reader: Place 100 µL of the cell suspensions into each of wells of a 96-well black microtiter plate. Monitor the fluorescence intensity (bottom read mode) with a fluorescence microplate reader at Ex/Em = 550/595 nm (Cutoff = 570 nm).

   Note If it is necessary to equilibrate the cell concentrations, adjust the suspension volume for the induced cells to approximate the cell density of the non-induced population. This adjustment step is optional if your cell treatment does not result in a dramatic loss in stimulated cell population numbers.

EXAMPLE DATA ANALYSIS AND FIGURES

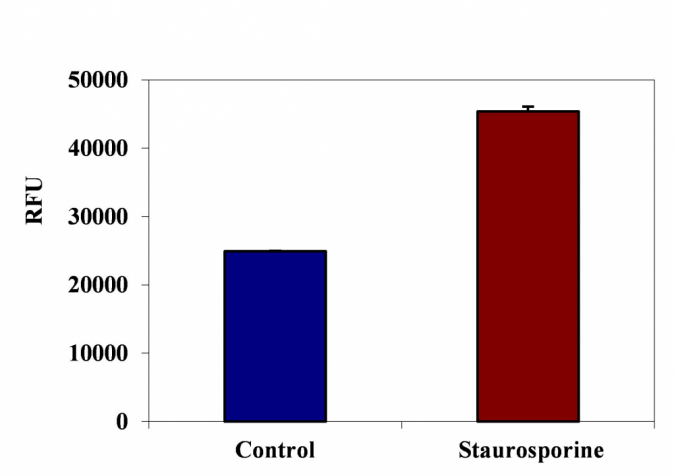

Figure 1. TF3-DEVD-FMK fluorometric detection of active caspases 3/7 using Kit #20101 in Jurkat cells. The cells were treated with 1 µM staurosporine for 3 hours (Red) while untreated cells were used as a control (Blue). Cells were incubated with TF3-DEVD-FMK for 1 hour at 37°C. The Fluorescent intensity (300, 000 cells/ 100 µL/well) was measured at Ex/Em = 550/595 nm (Cutoff at 570 nm) with a FlexStation microplate reader using bottom read mode.

DISCLAIMER